CELL DIVISION BY FURROWING IN MAGNOLIA

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In "Les centres cinétiques chez les Végétaux" (8), published in 1897, L. Guignard supported his previous contentions as to the existence of centrosomes in Angiosperms by studies on the reduction divisions of certain Dicotyledons, namely: Nymphaea, Nuphar, Limodorum, and Magnolia. Although most cytologists are unwilling to admit that Guignard succeeded in establishing his thesis, yet this must be recognized as a very critical piece of work, involving nearly one hundred careful drawings which bespeak excellent fixation. His figures represent stages in the division of the nuclei, and in addition some of those of Magnolia give stages in cytokinesis. There are included in the text a few paragraphs on cell division proper, but most emphasis naturally is placed on nuclear phenomena. In some of the figures there is shown an equatorial thickening of a few spindle fibers following the heterotypic karyokinesis, but other fibers seem to be uniform throughout. No reference is made in the discussion to the presence or absence of a cell plate. At a little later stage the fibers of the central spindle have a twisted, crinkled appearance in the equatorial region for about one third of their length. Meanwhile an equatorial furrow has developed for a short distance centripetally. It is shown in all stages of interkinesis to be of about uniform diameter at its base and rather sharp at the apex. Just before the nucleoli disappear in the beginning of the homoeotypic division the furrow reaches a depth about equal to the remaining isthmus, and it is said to remain arrested there throughout this mitosis. However, Guignard's figure 25 shows a middle anaphase with the furrow only one half as deep as stated. No figures are shown of the completion of the furrow nor of cytokinesis of any kind after the homoetoypic karyokinesis. It has thus not been fully established that this furrow is related to cell division, nor that there is no cell plate involved in the division of these cells. Guignard's figures represent the mother-cell wall as thickened to about the same extent as is shown by my drawings, although in the former it is of more nearly uniform thickness throughout. However,

in general the drawings of Guignard are in harmony with my own observations recorded below. There are nevertheless certain features in Guignard's work that require further consideration. One concerns the occurrence of a cell plate in the meiotic divisions of Magnolia, another the hour-glass spindle which is shown in his figure 21. In appearance the latter resembles somewhat an oblique section through a cell after the homoeotypic nuclear division is finished, showing two non-sister nuclei and the spindles between them which traverse the heterotypic equator. Since the paper by Guignard, two more publications upon the cytology of Magnolia have appeared. But Andrews (1) and Maneval (13) respectively contribute no additional points on these problems.

A recent paper (6) by the writer purported to establish quadripartition of the pollen mother cells of certain Dicotyledons by a process of furrowing rather than by the typical method of cell plate formation. Since the time of Strasburger's monumental work (15) in 1875, the division of all cells of the higher plants had been supposed to be by cell plates. A search through the literature, however, revealed a number of drawings which unwittingly on the part of their authors point to another interpretation. Furthermore, a study of six genera representing the Compositae, Primulaceae, Solanaceae, and Tropaeolaceae respectively, the last being the one in which Strasburger himself studied the pollen mother cells in this regard, convinced the writer that cell plates are not formed during the division of these cells. Nicotiana was the form most carefully investigated, and it was established that no cell division normally occurs between the first and second nuclear divisions, and that cytokinesis is accomplished by furrowing after the homoeotypic karyokinesis. At this time the four nuclei are tetrahedrally arranged in the cell and a spindle connects each pair of them. A furrow is formed along the equator of each of these six spindles. There are thus four points on the plasma membrane at each of which three furrows meet. At these points the depression of the plasma membrane toward the center of the tetranucleate cell is greatest. These four projections finally become united at the center of the cell, which thus becomes transformed into a four-lobed structure. The isthmuses connecting these lobes gradually become narrower until the division is complete, each lobe becoming one of the microspores. During this process the mother-cell wall swells and at all times fills the furrow, so that a layer of it lies between the microspores as soon

as they are formed. During this division of the cell there is no indication whatsoever of an equatorial differentiation in the spindle, nor does such occur immediately after the heterotypic nuclear division.

There is considerable resemblance between this particular mode of cytokinesis and the typical division of animal cells, except that it is quadripartition and not bipartition. There are also no centrosomes in these plant cells, unless the work of Guignard can be taken as conclusive. The conditions under which these pollen mother cells are formed are not unlike those of such animal cells as eggs, etc., but are quite different from those of most cells of higher plants. These cells are free-floating in the liquid of the anther instead of being in a compact tissue. Beer (3) considers that the walls of these mother cells are composed of pectose, which swells in water, becoming soft and gelatinous instead of being relatively rigid and non-elastic as are cellulose walls. Finally they assume a spherical shape instead of being pressed into parallelopipeds and other flat-faced forms by mutual pressure, as occurs in root tips and other parts of higher plants. This similarity in the conditions which surround the pollen mother cells and many animal cells led the writer to conclude that these conditions have some physicochemical effect in determining the type of cell division and that they might explain the resemblance of the pollen mother cells to the animal cells in this regard and their departure from the mode of cell division characteristic of the cells of most higher plants.

Material and Methods

The present study is based upon cultivated varieties of Magnolia growing at Cinchona Station on the island of Jamaica. Acknowledgments are due Columbia University for the William Bayard Cutting Traveling Fellowship, which made possible the collection of this material. The writer desires also to express his appreciation to Professor R. A. Harper, who offered many helpful suggestions during the prosecution of this work.

The methods employed are the same as were used in the writer's previous investigation (6). Flemming's strong chromic-acetic-osmic solution was used in fixation and his safranin-gentian violet-orange G combination was the stain employed. Living cells were also studied, especially those of *Magnolia tripetala*.

Living Cells

A study of the pollen mother cells of *Magnolia tripetala* L. was begun on April 21, 1917, and continued over the first of May upon material collected in the New York Botanical Garden. Before the initial date the buds and bud scales remained intact, and the buds were not enlarged much over their winter condition. As synapsis takes place, however, there is a very rapid increase in size, and the outer bud scales tear loose from their basilar attachment but remain coherent at their apices forming a sort of "calyptra" over the swelling bud. Not all the buds of the tree develop simultaneously. The external aspect of the bud in the above-noted particulars seems to indicate rather characteristically the exact cytological stage of the mother cells within. Just the relation of the swelling of the bud to the stages of reduction is not exactly clear. The anther swells more rapidly than do the pollen mother cells, so that the latter become suspended in the liquid of the pollen sac.

In living material the spindle fibers are easily discernible in the equators of the cells in the heterotypic division and also between the sister nuclei of the second mitosis. The fibers extending from the furrows to the nuclei are especially prominent. A few cases were noted in which an equatorial streak resembling a cell plate was apparent in the mother cell. In only one case was it found in a cell which also appeared to have furrows.

The usual number of microspores within a single mother-cell wall is four; but the occasional occurrence of supernumerary pollen grains is noted here as in several other flowering plants, notably Fuchsia (2) and Hemerocallis (10). One case was noted in Magnolia in which there were seven cells within the mother-cell wall, but none were found with more than this number. In several cases there were four microspores of normal size and one additional small one, or in some instances two such small cells.

Several stamens were studied with a view to determining the various ways in which the four microspores are arranged with respect to each other. Quantitative results were also obtained to determine the percentage of each type of arrangement. In Table I five types of arrangement are recognized. The accompanying drawings show the characteristic appearance of each type as seen through the microscope. Type 1 is the arrangement referred to by Giesenhagen (7)

as *decussate*, resulting from the spindles of the second meiotic division being at right angles to each other. Type 2 is the result of the spindles being parallel and may be called *quadrate*. Type 3 is the consequence of the two spindles being nearly at right angles to each other, and hence it is an approach to the first type. Type 4 is a similar approach to the second; and Type 5 is the characteristic tetrahedral disposition so common in many Dicotyledons. Types 6 and 7 (not represented by drawings) indicate mother-cells with four large cells and one small cell, and three large cells and one small cell respectively. The figures in the perpendicular columns refer to the number of pollen mother cells of single stamens, designated respectively as *A*, *B*, *C*, and *D*.

TABLE I

Arrangement of Microspores

Type	*A*	*B*	*C*	*D*	Total	Percent
1	34	43	35	35	147	51.2
2	20	15	22	14	71	24.75
3	11	8	13	5	37	12.9
4	4	6	8	5	23	8
5	0	0	0	0	0	0
6	1	3	0	4	8	2.8
7	0	1	0	0	1	.35
	70	76	78	63	287	100.00

These figures show that the tetrahedral arrangement of the microspores is exceedingly rare in Magnolia, if, in fact, it ever occurs at all. It also appears that the percentage of the various types of arrangement is rather markedly constant among various stamens, none of those studied departing very far from the mean. Approximately one half of the total number of cells in each stamen have the decussate arrangement, about one fourth have the quadrate, and deviations from these two types make up the remaining one fourth. In the study of the fixed material some data have been accumulated as to the relative positions of the two spindles during the homoeotypic karyokinesis. In 25 of 45 mother cells the spindles were nearly at right angles to each other. In the other 20 they were nearly parallel. Inasmuch as the latter arrangement is associated with the quadrate disposition of the microspores, it is interesting to note that in both cases these are in the minority respectively. This agreement between the arrangement of the microspores and the corresponding position of the spindles con-

firms the general opinion that the plane of cell division is determined by the direction of the spindle (Giesenhagen, 7), or, perhaps, that they are consequences of the same factors.

Regarding the formation of the furrow, the observations on living cells confirm the statement of Guignard (8) that an incipient furrow is developed during the first division, which is, however, arrested and only completed after the homoeotypic karyokinesis has taken place. In some cases the heterotypic furrow seems to be preceded by an equatorial differentiation, but this always disappears before cytokinesis is effected. During the homoeotypic nuclear division the heterotypic furrow remains arrested, but after the four nuclei are organized this furrow completes the division of the cell. At the same time the homoeotypic furrows are developed across the equators of the homoeotypic spindles at right angles to the heterotypic furrow, resulting in the mother cell's becoming subdivided into four microspores (fig. 10). Whether the heterotypic furrow completes the division of the mother cell into two parts before the homoeotypic furrow is complete is not easy to determine from the living cells, and consequently this question will be left until the study of the prepared slides is discussed.

There are certain terms that are necessary in referring to various parts of the mother cell during the reduction divisions; and it is desirable that the meaning of these words be clearly understood. The furrow which begins during the heterotypic division and is completed after the second mitosis is called the *heterotypic furrow*, even though the later stages of its development follow the homoeotypic karyokinesis. The spindle between the two daughter nuclei of the first division is called the *primary heterotypic spindle*; and the area which it crosses is the *heterotypic equator*. The spindle which crosses this equator after the second nuclear division is known as the *secondary heterotypic spindle*. The spindles connecting the sister nuclei of the second division are the *homoeotypic spindles*, and their equators are crossed by the *homoeotypic furrows*. The region of cytoplasm on the opposite side of each nucleus from the central spindle is the *polar region*, and that beside the nuclei and the spindles is the *lateral region*.

Nuclear and Cytoplasmic Phenomena

The stamen enlarges prior to the initiation of the reduction divisions, so that the pollen mother cells are free within the pollen sacs before

the leptoneme passes over into the pachyneme stage. In some cases the distance between the mother cells is once or twice their diameter, the individual cells being untouched by others on any side (fig. 12). In other instances they are found crowded together, especially in the ends or corners of the pollen sacs, so that each one touches other mother cells to the number of three to five (fig. 2). Even here the intercellular spaces are large (fig. 5), and the cells assume a spherical form. Relatively few cases are to be found in which a deviation from this shape seems to be due to crowding. Some cells in synapsis assume an elongated oval form, and occasionally the nucleus is found at one end of such a cell and the chromatin at the corresponding end of the nucleus. It is as if the chromatin thread had been attracted by something outside the cell and had moved in that direction, causing the nucleus also to migrate toward one end of the cell. Similar abnormal conditions are in evidence in the mitotic stages of these cells. It may very well be that these are wound effects, due to cutting the stamens from the flower before fixing them. Miehe (14) and Christman (4) found similar phenomena, such as the migration of the chromatin and nuclei from one cell into another, and suggested that they might be of a traumatic nature. R. S. Lillie (12) in 1903 found that free nuclei and those of sperms migrate toward the anode, but that cells with a large amount of cytoplasm go toward the cathode. Hardy (9) has more recently (1913) carried on further experiments on the effect of the electric current on cells, in general substantiating the conclusions of Lillie. It may be that these nuclear migrations, as the writer (6) has suggested is the normal procedure in Nicotiana, may be attributable to a disturbance in the electrostatic equilibrium of the cells of the tissue.

All of the pollen grains in a given anther are not in the same stage of the reduction divisions, which is a very helpful fact in the study of pollen formation. There are frequently found lying side by side cells in diakinesis and in the anaphases of the homoeotypic mitosis respectively. It is not, however, usual to find presynaptic stages and young microspores in the same pollen sac.

The stages of transformation within the nuclei are very marked and sharply defined in this material. There is an abundance of the bouquet, leptoneme, pachyneme (fig. 1), diakinesis, and the other phases of the meiotic divisions. No good contraction stages, however, are to be found, excepting the apparently traumatic effects referred to above. The description of the processes involved in the reduction of

the number of chromosomes in Magnolia is not given in this paper, but the series of stages are incidentally employed in determining the sequence of cytoplasmic phenomena. It is scarcely possible to doubt the validity of such stages as the formation of the dispireme (figs. 3 and 20), its transformation into the prochromosomes (figs. 3–5, 20–22), etc. The two series of nuclear and cytoplasmic changes seem to be remarkably constant in paralleling each other. Only during the interkinesis are any cases of possible lack of correspondence found. The photomicrographs and drawings given herewith show very clearly the simultaneous nature of these phenomena.

The number of chromosomes can be determined most easily by a study of the polar view of the metaphase of the homoeotypic division (fig. 9). The following are the results of counts made on a few cells: 45, 46, 45, 43. A fifth cell appeared to have more than any of these, but this was probably due to confusion of the two parts of the same chromosome and to difficulty in determining in each case whether they were pairs or only single chromosomes. The gametophytic number of chromosomes thus seems to be about 45.

The stages up to the telophase of the first meiotic division were described by Guignard (8) and will not be further discussed here. After the anaphases of the heterotypic karyokinesis the chromosomes assemble at the poles in the usual fashion and a nuclear membrane is formed, organizing a nucleus which is flat and discoid (figs. 2 and 26). The spireme is next formed from the chromosomes (figs. 3 and 20). At this time a streak appears midway between the two nuclei in exactly the position in which a cell plate might be expected. This streak takes the orange stain in Flemming's triple combination. Timberlake (16) in his study of cell division in the root tips of onion and the pollen mother cells of larch showed that such an "orange zone," as he called it, precedes the formation of the cell plate, and interpreted it as a preliminary step in cell-plate formation. In Magnolia it seems that the orange zone appears, but in no case is there any evidence of its being followed by the formation of a cell plate. It must be that the conditions for cell-plate formation obtain at first, but that they do not continue to exist, or that some factor enters in to interrupt the process. I was able to find this orange zone in only five or six out of fifty or more cells of Magnolia which, judging from the nuclear phenomena, were in exactly the same stage. It is thus by no means certain that it is formed in the division of every mother cell. Neither Andrews (1),

Maneval (13), nor Guignard (8) suggests even the possibility of the presence of such a structure. That it is in some instances formed cannot be doubted, as is shown by the photomicrograph (fig. 3) and the drawing (fig. 20) below. It is never seen to extend the entire distance across the mother cell, but in one or two instances the nuclei were closer to one side than to the other, and the orange zone reached the membrane on that side; in a few cases it was found to be interrupted, though this may have been caused by the shock of fixation. Cells in later stages can all be easily detected by the fact that the nuclei at the time of the presence of the orange zone are very flat and near together; whereas upon the disappearance of this body the nuclei round up and separate. Juel shows incomplete cell plates in Hemerocallis (10) and Carex (11), in the latter of which the cell plates are ephemeral. The case of Magnolia is slightly different, for it is not the cell plate which is ephemeral here, but rather the orange zone. Furthermore, in Magnolia the disappearance of this equatorial structure is followed by furrowing, which is not the case in the forms studied by Juel.

After the disappearance of the orange zone the nuclei enlarge and in them the nucleoli appear (figs. 4 and 21). The latter are at first small and there may be one or more of them in each nucleus, but they usually enlarge up to the time of the second division, keeping pace with the enlargement of the nucleus and even exceeding the latter in some instances. In fact, a nucleolus may become so large at times that it extends from the polar to the equatorial side of the nucleus (fig. 8). All except the large nucleoli are perfectly spherical. The latter often appear bell-shaped, like the starch grains of ginger. In addition to one or two large nucleoli in each nucleus at this stage there are also some small ones. A surprisingly large number of these cells in interkinesis have the large nucleoli exactly opposite each other in the nuclei of the same mother cell (figs. 6 and 21), though they may be in the center or at either side. Large nucleoli were found opposite each other in 59 cells, while in only 5 were they otherwise arranged.

While the nucleoli are enlarging, the furrow makes its appearance. It is difficult to arrange a series of stages in nuclear changes and furrow formation during interkinesis. However, the initiation of furrow formation is reasonably well indicated by nuclear phenomena. After the orange zone has disappeared the nuclei slowly enlarge and pull apart (fig. 5), becoming more nearly spherical (fig. 6). Before they attain the maximum distance apart the spireme has become completely

transformed into the prochromosomes of the resting nucleus. After nuclear migration has ceased and the nuclei have reached their maximum size, they again become much flattened at right angles to the direction of the heterotypic spindle (figs. 7 and 22). This is unquestionably in preparation for the homoeotypic karyokinesis (fig. 8). The breaking down of the nuclear membrane takes place before the disappearance of the nucleoli, and a multipolar spindle is organized about the chromosomes. These latter appear to arise directly by the enlargement of the prochromosomes and not by the interpolation of a spireme stage. Hence the prophase of the homoeotypic division is not exactly the reverse of the telophase of the heterotypic. As the multipolar stages progress the nucleoli continue to decrease in size, until by the time the bipolar spindles are formed they have completely disappeared. During the homoeotypic karyokinesis there is usually present an area of orange-staining homogeneous material on that side of each spindle that is toward the equatorial region of the previous division. It is often about equal to or wider than the spindle itself and extends for varying distances around to the polar side, but is always thicker toward the equator. In it no fibers are to be seen and the fibers in the region between the two orange areas become fewer and fewer as the prophases and metaphases progress. It is not improbable that the substance which composed the fibers of the primary heterotypic spindle becomes dispersed into the homogeneous orange-staining material. Only in rare instances is a fiber found crossing the heterotypic equator during the anaphases (fig. 9).

After the chromosomes are assembled at the poles in the telophases of the heterotypic karyokinesis, a nuclear membrane is formed in the usual fashion. No spindle fibers were found to be organized across the homoeotypic equator until this membrane appears (fig. 23). As the fibers make their appearance across the heterotypic equator the orange area above mentioned slowly vanishes. No orange streak or other equatorial differentiation is found in any of the spindles of the second mitosis. In fact, no cytoplasmic changes at all are distinguishable until the nuclei have enlarged (figs. 11 and 12) and the dispireme has gone over into the prochromosome stage. The furrows develop at this time (fig. 24). The details of this process will be discussed below.

The thickening of the wall is not as extreme as in Nicotiana (6), but is nevertheless very evident. It has the same staining reaction and homogeneous appearance as in the species of Dicotyledons for-

merly studied by the writer. In Magnolia, however, the thickening takes place more nearly uniformly over the entire surface of the cell, especially where the wall is not in contact with the walls of other cells (fig. 11). The writer does not find as great a degree of uniformity in the thickness of the cell wall as the figures of Guignard (8) indicate. It is ordinarily about one twenty-fifth of the diameter of the mother cell during the heterotypic division, and in interkinesis it thickens slightly, so that it becomes about one sixteenth to one twentieth of the diameter (figs. 13 and 23). During interkinesis the mother cells are in many cases elongated in the direction of the axis of the heterotypic spindle.

The Furrowing Process

In Nicotiana (6) there is neither a cell plate nor a furrow formed between the first and second nuclear divisions. In Magnolia, however, the formation of both of these structures is initiated, but neither is brought to completion before the homoeotypic karyokinesis. The former completely disappears, whereas the latter is arrested (figs. 6 and 8). Guignard's paper indicates that this furrow remains where it is arrested during the entire second division; but I am of the opinion (fig. 9) that, occasionally at least, it may recede somewhat. The furrow appears in the plasma membrane at the equator of the heterotypic spindle at a considerable interval of time after the stage of the ephemeral orange zone. Meanwhile the nuclei have enlarged and separated considerably (figs. 5 and 22), and the nucleoli have been developed. The nuclei remain in almost this same condition until they flatten in preparation for the next division. During this period of resting of the nucleus the furrow is being formed. It is obvious that there is some difficulty in arranging a series of stages in furrow formation on the basis of nuclear changes when there is no such series of collateral processes with which to compare it. One finds a great variety of furrows; some appear in section as minute mucronations, others are broad invaginations, some have sharp edges, others are rounded; some are shallow, others are deep. Any arrangement of these in a series must be merely hypothetical, but an attempt at seriation is not without value. It may be supposed that the furrow begins as a sharp, knife-like edge which deepens gradually and broadens at the base. The sharpness may be retained until the furrow reaches the maximum depth attained before the homoeotypic mitosis. There-

upon it probably becomes rounded at the end, of a more nearly uniform width, and finally may recede somewhat. In case it recedes, it doubtless becomes broadened to a greater extent (fig. 9). In some mother cells in the homoeotypic karyokinesis there appears to be very little if any furrow at all, whereas very few cells are to be found in which two fully developed nuclei occur without some sort of a furrow being present. All of which is evidence that a furrow is always formed during interkinesis and then may recede even to obliteration.

The cell-wall material always fills the furrow and appears as a jelly-like mass. The second division furrow is always narrow in section (figs. 13 to 18), and never broadens at the base like that of the first division. It is thus reasonable to assume that when the furrows begin to develop after the homoeotypic nuclear division, they progress continuously until the cell is divided, in this way differing from the first furrow which is known to be arrested in its development for a time. This sharp-edged furrow is the only kind that is to be found after the second division, and it is entirely probable that the heterotypic furrow begins in this way, as suggested above. If we think of the plasma membrane as the active agent in furrowing, and the attraction between it and the nuclear membranes as the force involved, this tension would be destroyed upon the disintegration of the nuclear membranes in the prophases of the homoeotypic karyokinesis, and the cell turgor would express itself in pressure against the plasma membrane, causing the equatorial furrow to recede or at least to flatten out somewhat. Conklin (5) and others have shown that in certain animal cells the cleavage furrows are arrested as a result of the cells being placed in hypertonic solutions, while the division of the nuclei and centrosomes may continue.

After the homoeotypic nuclear division, the daughter nuclei are at first narrow in section and close together (fig. 23), just as is the case after the first division. As the spireme passes over into the prochromosome stage the sister nuclei enlarge (fig. 12), become more nearly spherical (fig. 11), and pull apart slightly, but not so much as in the heterotypic division (fig. 15). No orange zone (fig. 23) or other semblance of an equatorial differentiation in the central spindle is found in the homoeotypic division. As soon as the nuclear membranes are formed, fibers appear across the heterotypic equator and soon definite spindles are organized. Because of the proximity and narrowness of the daughter nuclei, at first these spindles are not completely organized;

but later they are seen to be composed of fibers that run from each of the two sister nuclei on the one side to each of the two sister nuclei on the other. So that there are fibers running between every pair of nuclei in the tetranucleate cell, just as in the tetrahedral pollen mother cells of Nicotiana (6). In the latter case there are thus six distinct spindles, but in Magnolia there is some question as to whether they should be considered as six separate spindles or as two simple spindles and one compound spindle (figs. 14 and 24).

The formation of the spindle fibers in the heterotypic equator after the homoeotypic karyokinesis presents a problem which has as yet never been satisfactorily solved. Most central spindles are organized as a consequence of mitotic karyokinesis, just as occurs in the formation of the primary heterotypic and homoeotypic spindles in these cells. But in multinucleate cells that are formed by successive mitotic division of a primary nucleus, one or more spindles are apparently organized in some other way. In Nicotiana and several other Dicotyledons (6) four spindles are thus formed; in Magnolia there is one compound spindle so organized. In the last-named form this compound spindle lies in exactly the same place as did the primary heterotypic spindle, although the fibers in the two spindles are not arranged in the same way. The primary heterotypic spindle completely disappears as the homoeotypic division begins, and no semblance of it is found until after the new nuclei are formed. In the telophase a large number of fibers again appear. Whether these represent a reorganization of the primary heterotypic spindle, or the formation *de novo* of a large number of fibers is not entirely clear. The cells in the metaphase and anaphases show no spindle fibers (fig. 9) across this area. The cytoplasm in the equatorial region is granular, or in some cases apparently alveolar, and stains blue with the Flemming's treatment. The area of this type comprises about one third of the space between the two nuclei while the other two thirds between it and the two nuclei respectively is of a different composition. This latter takes on the orange stain and is homogeneous in appearance rather than granular. This orange area is widest on the equatorial side but extends around toward the opposite side for varying distances until it gradually disappears. No indications of fibers are to be found in this orange area, but it is not unlikely that it may be composed of material which once made up the fibers of the primary heterotypic spindle and which will again form the secondary one. In figures 24 and 25 of the writer's paper on

Nicotiana (6), it was shown that orange-staining granules apparently arrange themselves in rows to form the fibers of the new spindles after the second nuclear division. It is scarcely conceivable that so many fibers could be present in the cytoplasm as such during mitosis and not be detected. A greater probability seems to be that the material of the old spindle may have become dissipated throughout the cytoplasm and have formed the orange areas, later to be used again in forming the new fibers.

The furrows after the second division begin as sharp cutting edges formed by the infolding of the plasma membrane at its juncture with the equator of each spindle respectively (fig. 24). It will be observed that the secondary heterotypic spindle meets the plasma membrane at the summit of the heterotypic furrow, so that a new furrow is superimposed on the arrested one. The new one is always narrower than the older (figs. 14 and 24), so that their place of union remains marked throughout the process of furrowing. This superposition of the secondary heterotypic furrow on the primary one, as well as the usual elongation of the cell parallel to the heterotypic spindle, results in the equator of the latter being very narrow as compared with that traversed by the two homoeotypic spindles (figs. 13 and 25). In fact, the width of the secondary heterotypic spindle is frequently less than that of either homoeotypic spindle alone. Consequently, if all the furrows deepened with the same rapidity the cell would be divided into two parts first and then each of these would undergo bipartition by the homoeotypic furrows. This seems to be only rarely the case, and it is probable that the heterotypic furrow always develops somewhat more slowly than the homoeotypic furrows (fig. 15). In most instances there is apparently perfect quadripartition (fig. 17) due to the retardation of the speed of development of the secondary heterotypic furrow; but in a few cases (fig. 18) it is probable that the latter is completed slightly before the homoeotypic furrows have entirely closed their isthmuses.

Cases of normal cell plate formation are quite abundant in the cells composing the anther walls of these same stamens (fig. 19). It may thus be concluded that all cells of Magnolia except the pollen mother cells divide in this way. The plate appears in the center of the spindle first, as described by Strasburger (15) and Timberlake (16), and then develops centrifugally in the equator accompanied by the formation of new fibers and the widening of the spindle. This process continues

until the cell is completely partitioned, and the cell plate gives rise to plasma membranes between which the cell wall is formed. No indications of furrows or similar structures are discernible in these cells. It is observed that this cell plate formation occurs simultaneously with the organization of nuclear membranes and the union of the chromosomes to form the spireme. It is practically finished before the nuclei begin to enlarge and separate. The furrowing in both the heterotypic and homoeotypic divisions, on the other hand, is subsequent to and not simultaneous with these nuclear changes. It thus appears that the furrow and the cell plate are not homologous structures though they accomplish the same end. The formation of a furrow in the pollen mother cells must thus be regarded as taking place only when no cell plate has been previously formed. In other words, if a cell plate is for any reason not formed there is a furrow developed at a later time. The conditions incident to the two processes may not be at all the same. It is entirely likely that the conditions for furrowing exist in all cells of both plants and animals, but in plants they can not usually express themselves on account of the previous cell plate formation. Thus animal and plant cells may be potentially identical as to furrowing, but most plant cells in addition have the power of cell plate formation. The writer has previously suggested (6) that the latter process is related to the presence of a cellulose cell wall and the inability of the cell to enlarge in response to osmotic pressure, and hence is characteristic of plant cells alone.

The cause of the heterotypic furrow's remaining arrested until after the second nuclear division is a matter of considerable interest. The suggestion has been made by several writers that the lack of cytokinesis between the first and second karyokineses of the pollen mother cells of many plants is due to too short a period of interkinesis. The observations of the writer on Magnolia indicate that the period of interkinesis in this form is by no means short as compared with the time required for mitosis. The greater number of cells in my preparations were in interkinesis rather than in either the heterotypic or homoeotypic mitoses. If, as the writer has previously suggested (6), the furrowing is the consequence of a mutual attraction between the nuclear membranes and the plasma membrane, then an arrest of the furrow would be due either to a change in this force of attraction, or to the fact that the nuclei have such a size and position as to cause the resultant of the attracting forces to become zero after a certain

depth has been reached. Thus far no instance of bipartition by furrowing has been established in the higher plants, such as is so common in the animal kingdom. In the latter it is evident that the centrosomes are much smaller and much farther apart than are the nuclei in these mother cells, and hence if these centrosomes be the attraction centers, a resultant of forces might lead to complete bipartition. If, however, in these pollen mother cells the nuclei are the attraction centers, it may be that they are too large and too close together to accomplish complete partition in a binucleate cell. That the nuclear membranes are probably important in furrowing is indicated by the fact that furrowing is not resumed until the nuclear membranes are reformed in the telophases of the homoeotypic mitosis.

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EXPLANATION OF PLATES XXX–XXXI

Plates XXX and XXXI are photomicrographs of cells of Magnolia, all being pollen mother cells except figure 19. The magnification is 650, except in figures 10 and 19.

Plate XXXII presents drawings made with a Spencer microscope with tube length 17.5 cm., objective 1.5 mm. N. A. 1.30, and ocular 6 ×. Magnification 900.

PLATE XXX

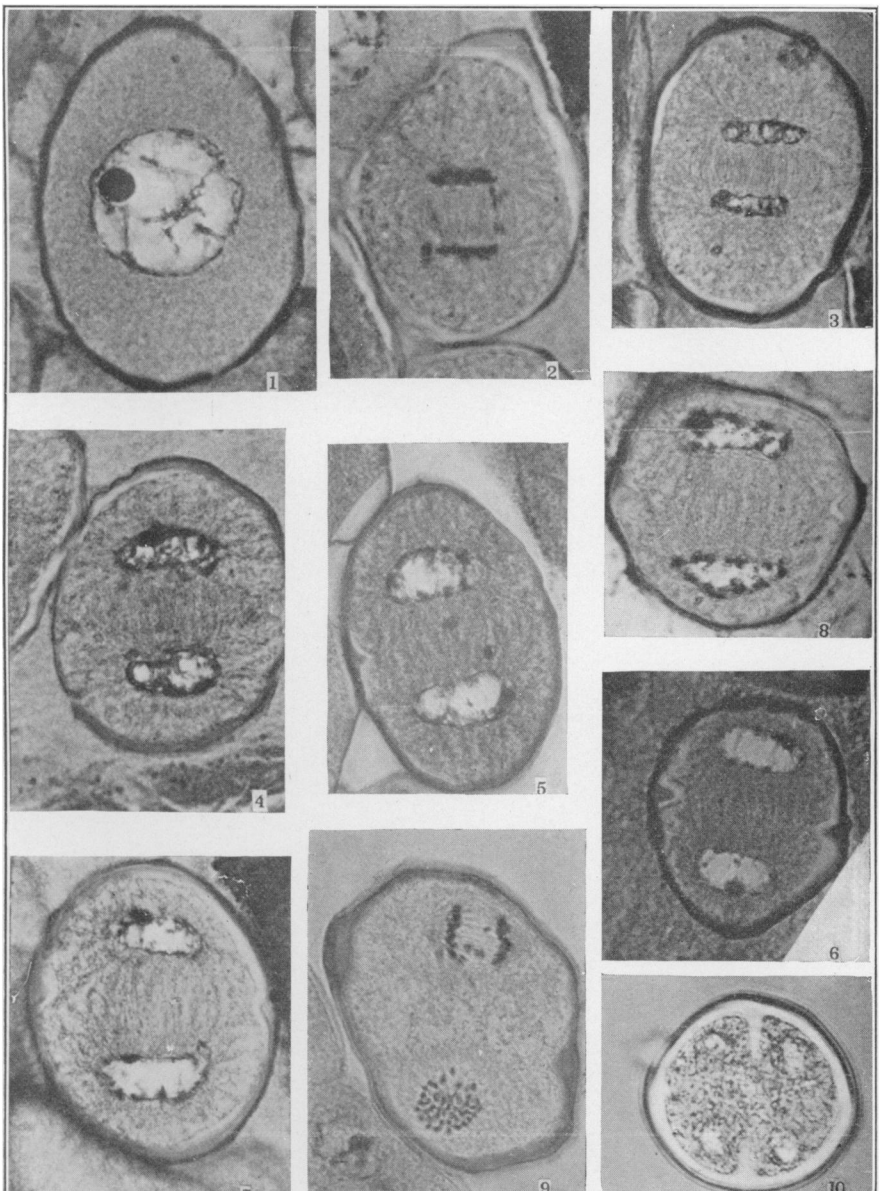
FIG. 1. Pachyneme of prophase of heterotypic mitosis.
FIG. 2. Early telophase.
FIG. 3. Nuclear membrane and orange zone forming.
FIG. 4. Nuclei larger, orange zone disappeared.
FIG. 5. Furrow beginning.
FIG. 6. Furrow well formed, nuclei in resting condition.
FIG. 7. Nuclei broader and larger.
FIG. 8. Nuclei very broad, nucleolus large.
FIG. 9. Anaphase of homoeotypic mitosis.
FIG. 10. Living cell showing furrows.

PLATE XXXI

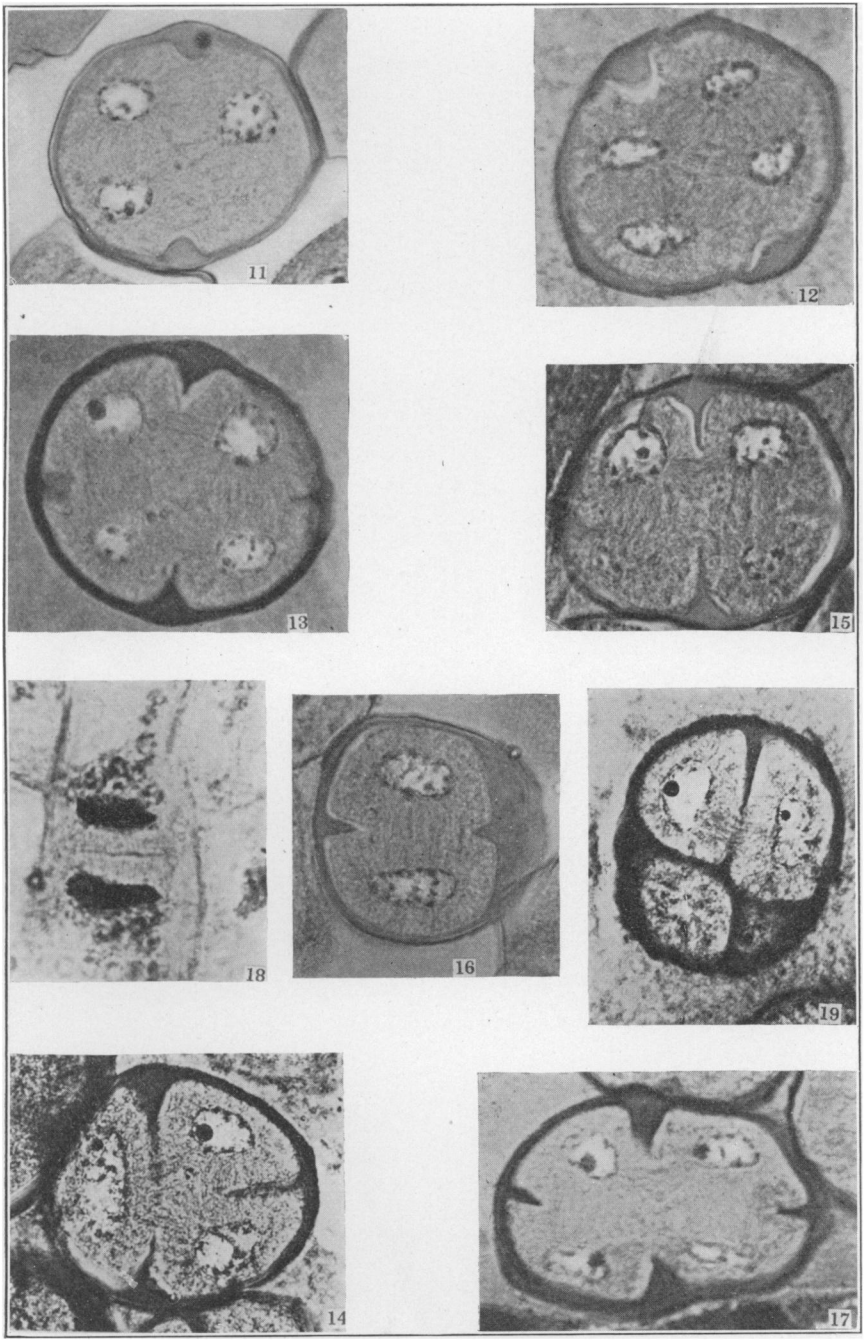
FIG. 11. Nuclei reorganized after second division.
FIG. 12. Same as Fig. 11, with spindles parallel.
FIG. 13. Same stage with cell spherical.
FIG. 14. Homoeotypic furrows forming.
FIG. 15. Furrow deep across heterotypic equator.
FIG. 16. Polar view of same stage as Fig. 13.
FIG. 17. Late stage in quadripartition.
FIG. 18. The completion of the furrow.
FIG. 19. Cell plate formation in a cell of the anther wall.

PLATE XXXII

FIG. 20. Dispireme stage following the heterotypic karyokinesis.
FIG. 21. After the orange zone has disappeared and before the furrow is formed.
FIG. 22. Furrow formation.
FIG. 23. Following the homoeotypic karyokinesis, no orange zone.
FIG. 24. Furrow being resumed.
FIG. 25. Furrows partially completed.



FARR: CELL DIVISION IN MAGNOLIA.



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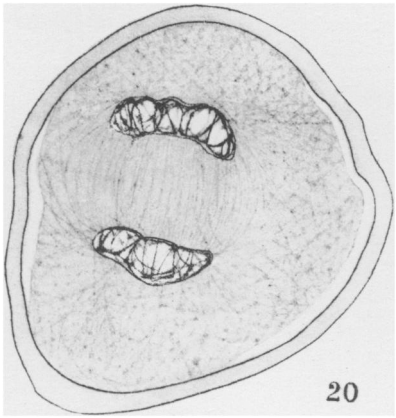

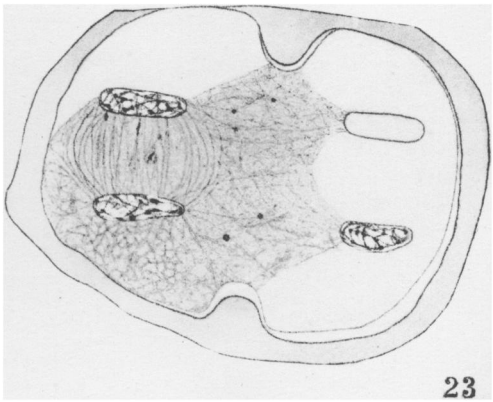

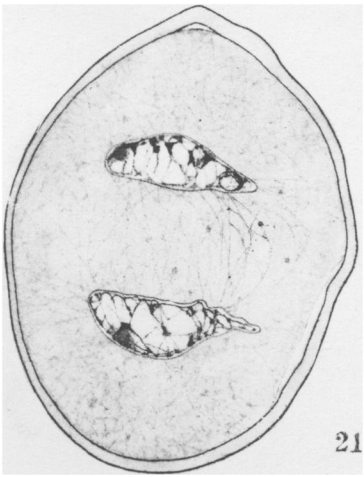

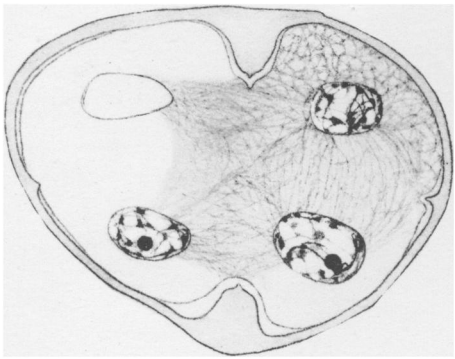

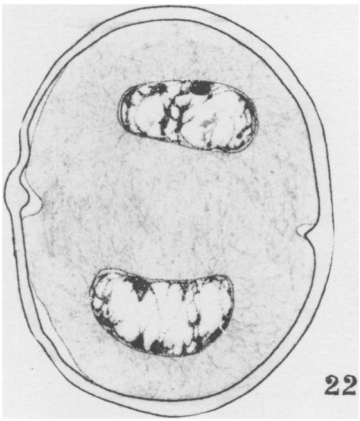

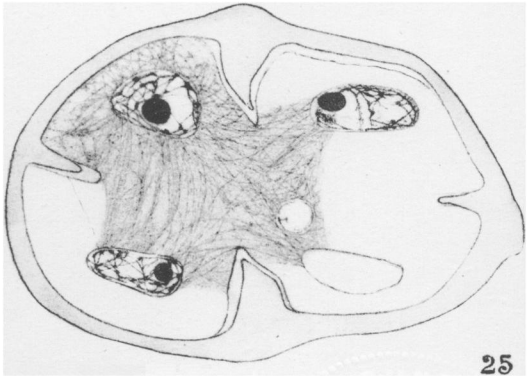

FARR: CELL DIVISION IN MAGNOLIA.